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LIPASE PRODUCTION BY *Aspergillus niger* GROWN IN DIFFERENT AGRO-INDUSTRIAL WASTES BY SOLID-STATE FERMENTATION

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Abstract – Filamentous fungi can easily degrade agro-industrial wastes in solid-state fermentation processes, synthesizing many important commercial biocompounds, such as lipolytic enzymes. The aim of this study was to evaluate the effect of the composition of the solid culture medium on the production of lipolytic enzymes by the fungus *Aspergillus niger.* Rice, wheat and soybean bran were mixed to prepare the culture medium, which was supplemented with glucose, glycerol or soybean oil. Four mixture experimental designs were used to find the best medium for enzyme production. According to our results, the highest lipolytic activity values were achieved with a mixture of rice bran and glycerol (19.844 $U \cdot g^{-1}$) or with rice bran only (13.267 $U \cdot g^{-1}$). Thus, the lipolytic enzyme could be produced without any additional carbon source apart from rice bran, although glycerol addition induced a higher production.

Keywords: Enzyme; Fungus; Agro-industrial wastes; Carbon source.

INTRODUCTION

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are enzymes that catalyze the hydrolysis and the synthesis of esters (Qamsari et al., 2011). Some lipases are capable of catalyzing the esterification, transesterification and interesterification of lipids (Burkert et al., 2004; Colla et al., 2010; Martins et al., 2008), water activity (a_w) being a determining factor in the reaction direction: forward (hydrolysis), equilibrium or reverse (synthesis) (Messias et al., 2011).

Lipases occur in plants, animals and microorganisms (Burkert et al., 2004; Martins et al., 2008; Roveda et al., 2010), but only microbial lipases can be produced in large-scale (Roveda et al., 2010; Qamsari et al., 2011). The great commercial importance of lipases is due to their wide range of applications in detergents, pharmaceuticals, foods (cheese and tea), pulp and paper, textiles, tanneries, cosmetics, biodiesel and wastewater treatment (Messias et al., 2011; Roveda et al., 2010; Yan et al., 2014).

Microbial lipases are mainly produced by submerged fermentation (SmF), which is a well-known operation in which the engineering aspects are currently fully developed. However, solid-state fermentation (SSF) has shown some advantages for enzyme production compared to SmF, even at a commercial scale (Thomas et al., 2013). It only needs a low percentage of free water to be conducted and it uses low cost substrates such as agricultural wastes, which act both as a source of nutrients for the fermentation process (Martins et al., 2008; Messias et al., 2011; Pandey, 2003),

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and as a support for the growth of microorganisms.

Solid-state fermentation studies involving different wastes and microorganisms have achieved good results with low enzyme production costs (Colla et al., 2010; Martins et al., 2008). Among these microorganisms, filamentous fungi are considered the most suitable for processes involving SSF due to their ability to grow with a low amount of free water (Castiglioni et al., 2009; Thomas et al., 2013; Vendruscolo et al., 2007) together with their efficiency to degrade some pollutants (Rigas et al., 2007; Ye et al., 2011). Particularly, the production of lipolytic enzymes by filamentous fungi in SSF from agro-industrial wastes has attracted great interest, since these enzymes can be easily extracted from the fermentation medium as they are mostly extracellular (Moura et al., 2013; Roveda et al., 2010).

Fungi of the genus *Rhizopus, Mucor, Rhizomucor, Geotrichum, Penicillium* and *Aspergillus* are reported to be lipase producing organisms (Mahadik et al., 2002; Mhetras et al., 2009). Among them, the fungus *Aspergillus niger* is considered to be promising for the synthesis of lipases with industrial applications (Mahadik et al., 2002), because of its ability to grow rapidly on a solid support, and synthesize large amounts of extracellular lipases (Kamini et al., 1998). Although studies involving optimization of enzyme production with different *A. niger* strains show some variations in their results, the presence of a lipid carbon source was shown to be essential for lipase production (Mahadik et al., 2002).

There are several variables that influence lipase production by filamentous fungi, such as the source of carbon and nitrogen, the composition and pH of the culture medium, the fermentation temperature, and the geometry of the bioreactor (Colen et al., 2006; Martins et al., 2008). Therefore, this study is aimed to evaluate the effect of the composition of the culture medium on lipase production by *Aspergillus niger* grown in SSF using agro-industrial wastes supplemented with different carbon sources.

MATERIALS AND METHODS

Microbial culture

The fungus used in this study was isolated from an oil sample from a vegetable oil refining company located at Gaspar–SC-Brazil. It was identified as *Aspergillus niger* by macro and micro-morphological analysis (Sidrim and Rocha, 2004; Zaitz et al., 2004) and tested for the production of lipase with different sources of nitrogen and carbon. Macro-morphological characteristics observed were: color, texture, relief and edges of the colonies.

To evaluate the micro-morphological characteristics, a micro culture technique was carried out in plates and the morphology of the hyphae of the yeast colony was analyzed. The plates were incubated for 5 days at 25ºC and then examined in an optical microscope (MOTIC, Q7720AD model). The fungus was stored in a medium containing potato dextrose agar (PDA), at 4°C and transferred to new dishes every 3 months.

Substrate and inoculum preparation

The inoculum was prepared according to a modification of the method of Pamboukian et al. (1998). Briefly, Erlenmeyer flasks of 250 mL containing 100 mL of Czapek culture medium (glucose, 30 $g \cdot L^{-1}$; $NaNO_3$, 2.0 $g \cdot L^{-1}$; K_2HPO_4, 1.0 $g \cdot L^{-1}$; $MgSO_4.7H_2O$, 0.5 $g \cdot L^{-1}$; KCl, 0.5 $g \cdot L^{-1}$; $FeSO_4.7H_2O$, 0.001 $g \cdot L^{-1}$; yeast extract, 1.0 $g \cdot L^{-1}$; and agar-agar , 20 $g.L^{-1}$) were seeded with a metal strap, and then incubated at 28°C for 7 days, until the surface had been completely covered and sporulation had occurred. Afterwards, 10 mL of a solution of TWEEN 80 (0.1% v/v) were added to obtain the spore suspension.

SSF assays were performed in cylindrical flasks of 500 mL with a perforated lid (1 inch in diameter) covered with filter paper. The culture medium consisted of 40 g of dry solid substrate (85 % bran and 15% of rice husk). Rice husk helps to increase porosity and average oxygen transfer. A nutrient solution containing KNO_3 (3.0 $g.L^{-1}$), $MgSO_4.7H_2O$ (0.5 $g.L^{-1}$), KH_2PO_4 (1.0 $g.L^{-1}$), peptone (0.3 $g.L^{-1}$) and yeast extract (1.0 $g.L^{-1}$), was added to the culture medium. The nitrogen sources tested were rice bran, wheat bran and soybean meal, supplemented or not with 1% of one of the following carbon sources: glucose, glycerol or soybean oil. The characterization of the agro-industrial waste used as the substrate (Table 1) was carried out according to the methods for food analysis of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008).

Table 1. Composition of agro-industrial wastes (w/w, %)

Parameters	Rice husk	Rice bran	Wheat bran	Soybean meal	Method
Carbohydrates	16.19	28.78	50.16	27.65	Fehling
Total ash	13.23	8.85	10.61	6.37	incineration
Ash soluble	12.81	7.31	9.35	2.55	incineration
Ash insoluble	0.42	1.54	1.26	3.82	incineration
Total Fiber	56.78	13.04	6.00	5.88	extraction in Soxhlet
Total Fat	0.60	27.60	4.48	1.74	direct extraction in Soxhlet
Proteins	2.56	12.53	18.71	46.44	Kjeldahl
Moisture (105ºC)	10.64	9.20	10.04	11.92	loss on drying
C/N	6.56	4.5	2.92	0.63	-

The culture medium was sterilized in an autoclave (20 *minutes* at *121ºC*) and the fermentation was then conducted for 7 days under the following conditions: 28°C, 50% moisture, and initial spore concentration of $4x10^6$ spores·g^{-1}. Moisture content, pH and water activity were measured at the beginning and at the end of the cultivation. All the assays and analytical determinations were carried out in triplicate.

The moisture content was determined by drying the samples of culture medium at 60ºC until constant weight, and calculated as the difference between the initial weight (wet mass) and the final one (dry mass) divided by the wet mass: $U(\%) = [(\text{weight}_{initial} - \text{weight}_{end}) / \text{weight}_{initial}] \times 100$.

Water activity in the culture medium was measured with the Aqualab® device (Decagon, USA) and the pH was determined by stirring 1 g of the medium in 10 mL of distilled water in a vortex mixer at maximum speed and measuring the pH of the supernatant with a pH meter (Tecnal, Brazil).

Enzyme extraction

Fermented solid medium (25 g) was mixed thoroughly with 100 mL of distilled water (pH 6.8) and shaken (160 rpm) for 60 minutes on an orbital shaker at room temperature. The extract was then filtrated through a 0.45 µm filter. The cell-free supernatant was used for the lipolytic activity assay (modified from Colen et al., 2006).

Enzyme assay

Lipase activity was determined by using the modified titrimetric method of Burkert et al. (2004) based on the titration with 0.05 N NaOH of the fatty acids released from the hydrolysis of olive oil, previously emulsified with xanthan gum, by the action of the lipase present in the fermentation extract. The lipase activity was calculated as described by Cerqueira (2007). One unit of lipase activity was defined as the amount of enzyme that releases 1 µmol of fatty acid per minute. The enzyme activity was expressed per unit dry mass of fermented medium (U·g^{-1}).

Experimental Design

In order to determine the optimum composition of the culture medium for lipase production a mixture experimental design was used. The design included seven experiments, each one corresponding to different amounts of rice, wheat and soybean meal in the medium, as shown in Table 2. As response variables lipase production, moisture content, water activity and pH were chosen. The experimental planning in Table 2 was conducted using an additional carbon source (glucose, glycerol or soybean oil) each time. A control design, with no additional carbon source, was also performed. All the assays were performed in triplicate.

Table 2. The experimental matrix of mixture design

Assays	Components (**weight fraction**)*		
	Rice bran	Wheat bran	Soybean bran
1	1	0	0
2	0	1	0
3	0	0	1
4	1/2	1/2	0
5	1/2	0	1/2
6	0	1/2	1/2
7	1/3	1/3	1/3

*Each experiment contained 6 grams of rice husk in its composition

The results were fitted to a cubic response surface model (Eq. 1) which was then used to optimize the fraction of each component in the final mixture.

$$Y = \beta_r x_r + \beta_w x_w + \beta_s x_s + \beta_{r,w} x_r x_w + \beta_{r,s} x_r x_s + \beta_{w,s} x_w x_s + \beta_{r,w,s} x_r x_w x_s \quad (1)$$

where Y stands for the response variable; x_r, x_w, and x_s are the weight fraction of rice, wheat and soybean brans, respectively; and β represent the model coefficients associated with each factor or their interactions.

Regression analysis was carried out in order to fit the mathematical model to the experimental data and to identify the best region for enzyme production. To do this the Statistic software© 7.0 (Stat Soft, Inc. Tulsa-OK, USA, license number 1.02.01.032/6718) was used.

RESULTS AND DISCUSSION

Water activity, pH, and moisture of the culture medium

Moisture content remained stable until the end of the fermentation (50 ± 0.02%) in all the assays, allowing the growth of the fungus. According to Pandey (2003), high moisture contents can reduce the porosity of the substrate, preventing the penetration of oxygen and facilitating bacterial contamination. However, low moisture content can lead to poor access to nutrients, causing a slow microbial growth. The moisture around 50% provided strong positive fungal growth. Studies on *A. niger* for lipase production by Santos et al. (2012) show a significant influence of the initial moisture content at temperatures similar to those used in this work.

Apart from moisture, pH and water activity (a_w) also exert a decisive influence on microbial metabolism. Water

activity is a fundamental parameter in the mass transfer of water and nutrients through the microbial cells (Pandey, 2003; Ramos-Sánchez et al., 2015). The values of a_w and pH at the beginning and at the end of the fermentation are listed in Table 3.

Table 3. Values of aw and pH at the beginning (0 day) and at the end (7 days) of the fermentation with different sources of nitrogen and carbon (mean ± standard error). Equal letters within the same test (same assay and carbon source) denote that no significant differences were found at a 5 % significance level.

Assay	Carbon Sources							
	Glucose		Glycerol		Soybean Oil		Only Bran	
	Water activity values							
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	0.989±0.001[a]	0.973±0.004[b]	0.974±0.001[a]	0.973±0.004[a]	0.993±0.001[a]	0.963±0.003[b]	0.988±0.001[a]	0.974±0.003[b]
2	0.979±0.001[a]	0.977±0.00[a]	0.971±0.001[a]	0.978±0.001[a]	0.979±0.001[a]	0.977±0.002[a]	0.982±0.001[a]	0.979±0.003[a]
3	0.967±0.002[a]	0.941±0.003[b]	0.964±0.001[a]	0.948±0.004[b]	0.981±0.001[a]	0.938±0.001[b]	0.968±0.001[a]	0.940±0.002[b]
4	0.985±0.001[a]	0.979±0.003[a]	0.980±0.001[a]	0.981±0.003[a]	0.983±0.002[a]	0.979±0.004[a]	0.977±0.001[a]	0.976±0.001[a]
5	0.979±0.001[a]	0.960±0.001[b]	0.975±0.001[a]	0.954±0.001[b]	0.982±0.001[a]	0.954±0.001[b]	0.974±0.001[a]	0.954±0.002[b]
6	0.973±0.001[a]	0.954±0.003[b]	0.970±0.001[a]	0.954±0.002[b]	0.972±0.001[a]	0.949±0.004[b]	0.969±0.001[a]	0.953±0.003[b]
7	0.979±0.002[a]	0.965±0.001[b]	0.972±0.001[a]	0.961±0.001[b]	0.977±0.002[a]	0.960±0.004[b]	0.975±0.001[a]	0.961±0.001[b]
	pH values							
1	6.2 ±0.014[a]	3.2 ±0.035[b]	6.4 ±0.014[a]	3.4 ±0.049[b]	6.5 ±0.028[a]	3.0 ±0.042[b]	6.1 ±0.035[a]	3.2 ±0.042 [b]
2	5.9 ±0.007[a]	4.4 ±0.028[b]	6.1 ±0.007[a]	5.5 ±0.021[b]	6.0 ±0.035[a]	5.3 ±0.042[b]	6.1 ±0.035[a]	5.4 ±0.035 [b]
3	6.1 ±0.007[a]	5.3 ±0.021[b]	6.6 ±0.014[a]	5.7 ±0.049[b]	6.5 ±0.007[a]	5.5 ±0.049[b]	6.6 ±0.021[a]	5.2 ±0.042 [b]
4	6.1 ±0.007[a]	4.2 ±0.014[b]	6.1 ±0.02[a]	3.9 ±0.042[b]	6.2 ±0.021[a]	4.0 ±0.049[b]	6.1 ±0.021[a]	4.4 ±0.014 [b]
5	6.2 ±0.021[a]	5.3 ±0.028[b]	6.5 ±0.021[a]	5.2 ±0.042[b]	6.4 ±0.021[a]	4.9 ±0.028[b]	6.2 ±0.021[a]	5.3 ±0.021 [b]
6	6.2 ±0.021[a]	5.1 ±0.042[b]	6.2 ±0.014[a]	5.3 ±0.028[b]	6.2 ±0.007[a]	5.1 ±0.007[b]	6.1 ±0.021[a]	5.1 ±0.021 [b]
7	6.1 ±0.014[a]	5.5 ±0.049[b]	6.4 ±0.014[a]	5.3 ±0.042[b]	6.4 ±0.014[a]	4.9 ±0.057[b]	6.1 ±0.028[a]	5.3 ±0.049 [b]

The a_w was reduced after 7 days of culture in most of the assays, particularly when the culture medium contained soybean meal, which may be caused by the consumption of water and nutrients by the fungus, to promote microbial growth. Significant reduction of the pH was observed in all the assays, with the largest reductions in media containing rice bran. The control experiment (containing rice husk and a salt solution only) showed no significant variation at a 95% confidence level, as follows: $a_{w\ initial} = 0.995 \pm 0.001$ and $a_{w\ end} = 0.991 \pm 0.001$; $pH_{initial} = 4.9 \pm 0.021$ and $pH_{end} = 4.4 \pm 0.042$.

Lipase production

Lipase production was observed in assays with rice bran, wheat bran or mixtures of both, but no positive results were found when soybean meal was present in the fermentation medium, as shown in Figure 1.

The contour plots indicate that rice bran promotes lipase production in all assays, obtaining the highest lipase activity among all the substrates tested. Enzyme production was also observed in assays with wheat bran and mixtures of the rice and wheat brans. Wheat bran showed favorable results when supplemented with glucose and soybean oil, but to a lower extent. Plots A and C in Figure 1 indicate that the percentage of wheat bran should not be higher than 25%, since lipase production decreased above this value. The addition of soybean oil to the medium (Graph D), favored the production of the enzyme in media containing only rice bran or wheat bran, but in smaller quantities. Soybean oil had no effect on lipase production in the experiments performed.

The control medium (containing rice husk and a salt solution only) showed no enzymatic activity under the conditions tested in this study.

Contour plots in Figure 1 A to D were generated using Equations 2 to 5, respectively. These equations were obtained by simplex-centroid regression and must be used with the codified values of the factor levels (Table 2). Only significant terms at 95% confidence levels are included. The coefficients of determination for each equation (from 2 to 5) were 99.98; 99.97; 99.98 and 99.88% respectively.

$$Only\ bran = [13.266\ x_r + 8.133\ x_r x_w - 26.533\ x_r x_s - 64.200\ x_r x_r x_s] \quad (2)$$

$$Glucose = \begin{bmatrix} 12.0233\ x_r + 10.2433\ x_w - 1.9466\ x_r x_w - 24.046\ x_r x_s \\ -20.4866\ x_w x_s - 60.960\ x_r x_w x_s \end{bmatrix} \quad (3)$$

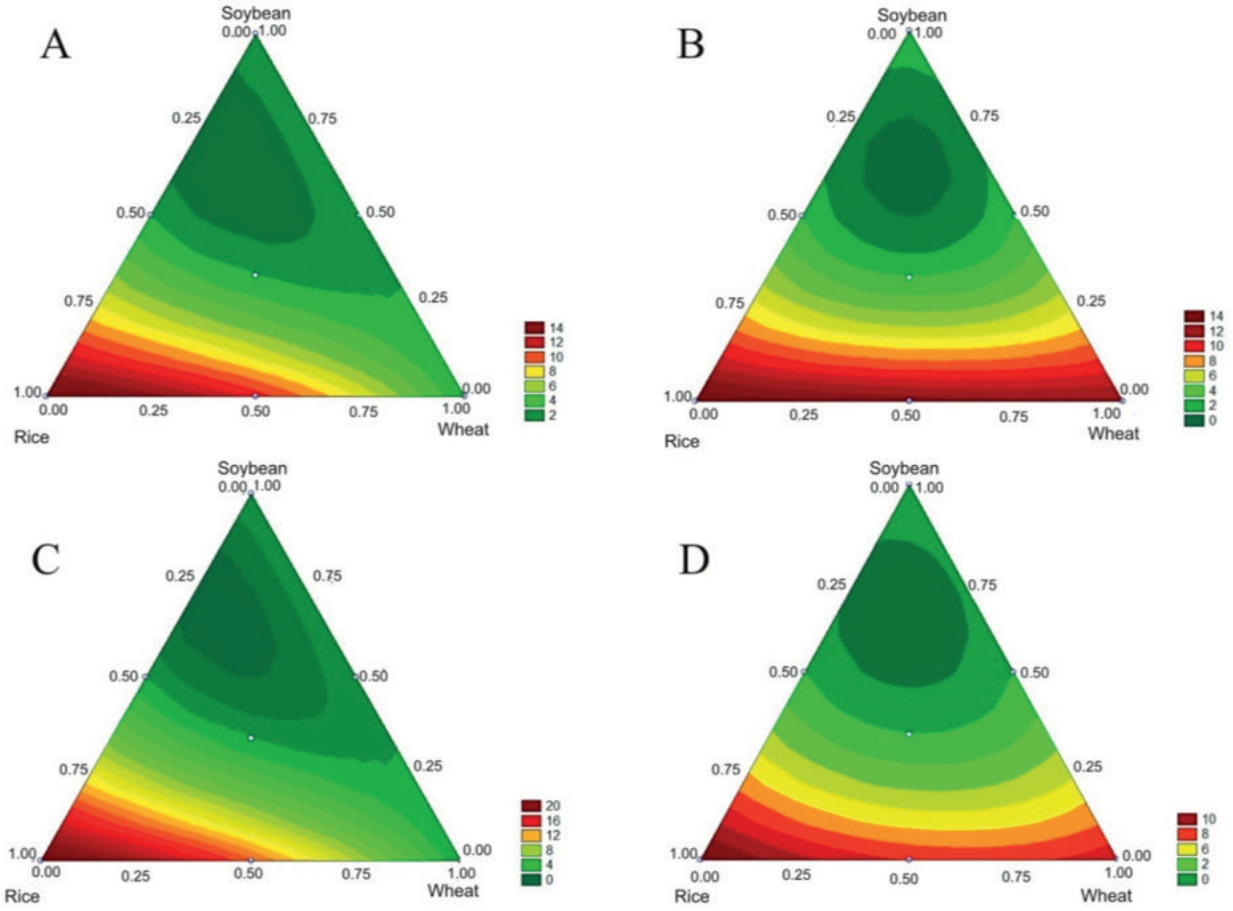

Figure 1 - Contour plot for lipase production using different carbon sources: **A** bran not supplemented with carbon source (rice, wheat and soybean); **B** bran (rice, wheat and soybean) supplemented with glucose**; C** bran (rice, wheat and soybean) supplemented with glycerol; **D** bran (rice, wheat and soybean) supplemented with soybean oil.

$$Glycerin = [19.8466\, x_r + 5.3733\, x_r x_w - 39.6933\, x_r x_s - 75.660\, x_r x_w x_s] \quad (4)$$

$$Soybean\ oil = \begin{bmatrix} 9.9766\, x_r + 6.78\, x_w - 9.7\, x_r x_w - 19.9533\, x_r x_s \\ -13.56\, x_w x_s - 21.17\, x_r x_w x_s \end{bmatrix} \quad (5)$$

The experimental lipolytic activity and that calculated through the equations above are shown in Table 4.

Lipase activities for each test are compared in Figure 2.

The maximum lipase yield (19.844 $U \cdot g^{-1}$) was achieved using rice bran and glycerol as supplementary carbon source, followed by rice bran without supplementation (13.267 $U \cdot g^{-1}$), as can be seen in Table 4. The experimental values exhibit an excellent correlation with the predicted ones in all assays performed, confirming that the simulation produces results that are realistic and make sense.

The large amount of protein present in soybean bran may have induced the fungus to synthesize higher amounts of protease during fermentation, somehow decreasing lipase production. Other researchers have also attributed the decrease in lipolytic activity in the fermentation to protease production (Palma et al., 2000; di Luccio et al, 2004). However, the best lipase producing microorganisms also produce protease (Martins et al, 2008). This suggests that protease production may have affected the stability of the lipase; however, further experiments on the determination of proteolytic activity are necessary to confirm this hypothesis.

Table 4. Lipase activity obtained after 7 days of fermentation

Assay	Treatment	Lipase activity (U·g^{-1})			
		Glucose		Glycerol	
		experimental	calculated	experimental	calculated
1	(1; 0; 0)	12.022 ± 0.047	12.023	19.844 ± 0.236	19.846
2	(0; 1; 0)	10.200 ± 0.283	10.243	NA	NA
3	(0; 0; 1)	NA	NA	NA	NA
4	(½; ½; 0)	10.633 ± 0.047	10.646	11.266 ± 0.094	11.266
5	(½; 0; ½)	NA	NA	NA	NA
6	(0; ½; ½)	NA	NA	NA	NA
7	(1/3; 1/3; 1/3)	NA	NA	NA	NA
Assay	Treatment	Soybean Oil		Only Bran	
		experimental	calculated	experimental	calculated
1	(1; 0; 0)	9.967 ±0.047	9.976	13.267 ± 0.057	13.266
2	(0; 1; 0)	6.767 ± 0.141	6.780	NA	NA
3	(0; 0; 1)	NA	NA	NA	NA
4	(½; ½; 0)	5.967 ± 0.047	5.953	8.633 ± 0.047	8.666
5	(½; 0; ½)	NA	NA	NA	NA
6	(0; ½; ½)	NA	NA	NA	NA
7	(1/3; 1/3; 1/3)	NA	NA	NA	NA

*NA they did not show lipase activity in the applied tests. Mean ± standard deviation of the three replicates. Treatments in sequence: bran (rice, wheat, soybean)

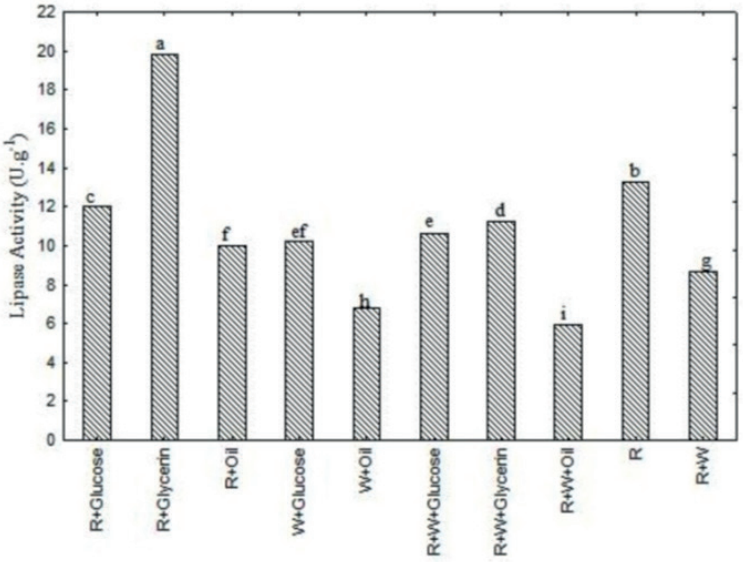

Figure 2 – Lipase activity in U·g^{-1} after fermentation with different nitrogen and carbon sources: R: rice and W: wheat. Same letter denotes statistically equal treatments.

Similar values of lipase production were also reported by Di Luccio et al. (2004) who cultivated *Penicillium simplicissimum* in SSF using soybean cake and olive oil as inducer, the maximum lipase activity (21 U·g^{-1}) being attained after 94 hours of cultivation at 27°C and with 4% olive oil.

Sun and Xu (2008) also studied lipase production with the fungus *Rhizopus chinensis* using flour and wheat bran as substrate (3:2 w/w). After 72 hours of incubation, the maximum lipase activity (24.44 U·g^{-1}) was obtained under the following conditions: 70% moisture, pH 6.5, with peptone (2% w/w) and olive oil as inducer (2% v/w).

Determination coefficients (R^2) were significant ($p<0.05$) in all lipase production assays. Results in Equations 2, 3, 4 and 5 clearly show that the soybean bran did not significantly contribute to enzyme production. Thus, the lipase production depends only on rice and wheat bran. However, wheat bran was effective only when supplemented with additional carbon sources (glucose and soybean oil), as shown in Figure 1. Model coefficients with

a negative value indicate an antagonistic effect on enzyme production of the combination of the corresponding carbon or nitrogen sources.

This can be explained by the C/N ratios (total) of the brans under study, which were 4.5, 2.92 and 0.63 respectively for rice, wheat and soybean (calculated from the results in Table 1) since the C/N ratio of the substrate has a significant influence on the production of enzymes (Rughoonundun et al., 2012). A high C/N ratio is deficient in nitrogen and slows the digestion rate because there are insufficient cells to maintain active microbial biomass. A low C/N ratio has a high nitrogen content, so ammonia can be produced, which can completely halt the fermentation (Rughoonundun et al., 2012) due to its toxicity.

The C/N ratio of the rice bran used in the experiments is adequate to promote both fungus growth and lipase synthesis without the need for an additional carbon source. On the other hand, wheat bran did not contribute to lipase production except in the presence of a supplementary carbon source (glucose and soybean oil).

Nitrogen sources (organic and inorganic) used to supplement the culture medium greatly influence lipase production (Ramos-Sánchez et al., 2015). Agro-industrial wastes, besides serving as a support for microbial growth, serve as a source of nitrogen. In many cases, only the waste is sufficient to meet the microbial needs with respect to nitrogen for growth and enzyme synthesis (Ramos-Sánchez et al., 2015). For the cultivation of *Aspergillus niger*, rice and wheat bran have served as an effective source of nitrogen.

Nitrogen content of soybean bran used in this study resulted in a higher mycelial growth, no enzymatic activity being obtained with this substrate. The concentration of carbon and nitrogen sources are among the important process parameters that have been studied and optimized (Ramos-Sánchez et al., 2015).

The suitability of rice bran for lipase production may be attributed to the oil present in it. This oil may have induced the production of this enzyme without an additional carbon source, since the amount of fatty acids present in the rice bran (27.60%) may have been sufficient to induce the production of lipase in the fermentation process. When soybean oil was added to rice bran, lower lipolytic activity (9.967 $U \cdot g^{-1}$) was obtained. Supplementation of glycerol in half fermentation favored enzyme production in this study.

Pure and residual glycerol have been used in the production of some bioproducts, including citric acid (Levinson et al., 2007), erythritol (Rymowiez et al., 2009), 1,3-propanediol and single cell oil (Papanikolau et al., 2008). However, few studies have been published aimed at the production of enzymes such as lipases, using glycerol as a carbon source. Studies by Rech (2010) using *Staphylococcus warneri* and glycerol as a carbon source showed excellent results regarding enzymatic activity. Volpato et al. (2008) also obtained satisfactory lipase production results with *Staphylococcus caseolyticus* EX17 growing on crude glycerol obtained from biodiesel synthesis.

Colla et al. (2010) made a comparative study of lipase production by submerged fermentation and solid-state fermentation using *Aspergillus sp.* In SSF they used soybean bran and rice husk supplemented with a nutrient solution and added olive oil and sodium nitrate as carbon and nitrogen sources respectively. The enzymatic activity values obtained were higher in SSF than in SmF. The same behavior was observed in studies by Sundar and Kumaresapillai (2013) using *Aspergillus niger*. This shows the potential of SSF for the production of enzymes.

CONCLUSIONS

Agro-industrial wastes have been used as carbon source for microbial growth and lipase production by *Aspergillus niger*, a fungus which belongs to a prominent and important genera of filamentous ascomycetes. The mixture of husk and rice bran proved to be the most appropriate medium for lipase production, especially when it was supplemented with glycerol. Therefore, this mixture can be used as feedstock for lipase production by solid-state fermentation. Wheat bran, when supplemented with glucose, was also found to be suitable for this purpose. Mixture experimental design proved to be a powerful tool for finding the optimal composition of the culture medium in the production of lipolytic enzymes by *Aspergillus niger*, which is a very important aspect for the development of SSF processes. The excellent correlation found between experimental and predicted data confirmed the validity of the model.

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